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(54) OSTEOINDUCTIVE PHARMACEUTICAL FORMULATIONS

OSTEO-INDUKTIVE PHARMAZEUTISCHE FORMULIERUNGEN

COMPOSITIONS PHARMACEUTIQUES OSTEOINDUCTIVES

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- **CHEMICAL ABSTRACTS, vol. 75, no. 7, 16 August 1971, (Columbus, Ohio, US), A.G. Korobkina et al.: "Action of epsilon-aminocaproic acid during combined radiation injuries", see page 60, abstract 45404a, & Voenno-Med.Zh. 1971, (2), 42-6**

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Description

This invention relates to cartilage and/or bone inductive pharmaceutical formulations. More specifically, it relates to the use of epsilon aminocaproic acid (EACA) or other lysine analogues, serine protease inhibitors or other antifibrinolytic agents in cartilage and/or bone inductive formulations.

Formulations of the invention comprise EACA or other lysine analogues, serine protease inhibitors or antifibrinolytic agents in conjunction with cartilage and/or bone inductive proteins such as BMP-2 (having been designated in the past as BMP-2A or BMP-2 Class I), BMP-3, or BMP-4 (having been designated in the past as BMP-2B and BMP-2 Class II) disclosed in PCT International Publications WO88/00205 and WO89/10409. Further cartilage and/or bone inductive proteins for use in the invention include BMP-5, BMP-6, and BMP-7 disclosed in PCT International Publication WO90/11366.

In further embodiments the formulations may further comprise growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factors (TGF-α and TGF-β), and platelet derived growth factor (PDGF).

The formulations may also include an appropriate matrix, for instance, for sequestering and/or support of the composition and/or providing a surface for bone and/or cartilage formation. The matrix may provide sequestering of the cartilage and/or osteoinductive protein(s) and/or the appropriate environment for presentation of the protein(s).

EACA is the presently preferred lysine analogue for use in formulations of the invention. The formulation may comprise other lysine analogues including trans-p-aminomethyl-cyclohexanecarboxylic acid (AMCA; tranexamic acid) (Amstat). The presence of EACA in osteoinductive formulations accelerates the amount of bone formation and/or decreases the amount of cartilage and/or bone inductive protein required.

EACA is known to have a fibrin stabilizing effect. It inactivates plasmin which is a serine protease. [Nilsson et al Lancet 1: 1233-1236 (1960)]. EACA has been shown to enhance new collagen synthesis in animals through blockage of the fibrinolytic system. [Brandstedt et al, Eur. Surg. Res. 12: 12-21 (1980)]. We have found that EACA increases the sensitivity of osteoinductive assays by accelerating the amount of cartilage and/or bone formed and/or decreasing the amount of cartilage/bone inductive protein required.

The invention further features a method for formulating the compositions of the invention, as well as use of the agents for preparing pharmaceutical compositions for treating a number of bone and/or cartilage defects, and periodontal disease. The formulations may also be employed in methods for treating various types of wounds and in tissue repair. The compositions of the invention are administered to a patient needing such bone and/or cartilage formation, wound healing or tissue repair. The method therefore involves administration of a therapeutically effective amount of a lysine analogue, serin protease inhibitor, or other antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive protein in a pharmaceutically acceptable carrier. These proteins include, for instance at least one of the "BMP" proteins disclosed in the co-owned applications described above.

In addition, these methods may further entail administration of other growth factors including EGF, FGF, TGF-α, TGF-β, and PDGF.

Other aspects and advantages of the invention will be apparent based upon consideration of the following detailed description and preferred embodiments thereof.

Detailed Description of the Invention

The osteoinductive formulations of the invention comprise EACA, a lysine analogue, in conjunction with a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle. Other synthetic lysine analogues which may be used in practice of the invention include trans-p-aminomethyl-cyclohexanecarboxylic acid (AMCA; tranexamic acid). Inhibitors of fibrin clot lysis, other than the lysine analogues mentioned above, for instance serine protease inhibitors, in conjunction with a cartilage and/or bone inductive factor may also comprise formulations of the invention. Such serine protease inhibitors may include aprotinin, α_2 antiplasmin and α_2 macroglobulin. A further fibrinolytic agent which may be useful in formulations of the invention is p-amino methyl benzoic acid.

The cartilage and/or bone inductive factors which may be used in formulations of the invention include, but are not limited to BMP-2, BMP-3 and BMP-4 disclosed in International Publications WO88/00205 and WO89/10409. Further cartilage and/or bone inductive proteins for use in the invention include BMP-5, BMP-6, and BMP-7 disclosed in PCT International Publication WO90/11366.

The use of EACA in osteoinductive formulations accelerates the amount of bone formation and/or decreases the amount of cartilage and/or bone inductive protein required. For example, with EACA present significantly more bone formation is seen at seven days post implantation using the same amount of bone inductive factor as compared to formulations without EACA.

In addition, the formulations of the invention may further comprise autologous blood. At the time of surgery the matrix (described below) is mixed with a sufficient quantity of the patients' blood, EACA and cartilage/bone protein. The suitability of autologous blood is based on its biocompatability and ready availability. Autologous blood may also be utilized instead of another matrix. Further formulations of the invention comprise EACA (or other fibrinolytic agents) autologous blood and cartilage/bone inductive protein.

In addition to the cartilage/bone protein, the formulations may include at least one other therapeutically useful agent including growth factors such as epidermal

growth factor (EGF), fibroblast growth factor (FGF), transforming growth factors (TGF-α and TGF-β), and platelet derived growth factor (PDGF).

The formulations may also include an appropriate matrix, for instance, for delivery and/or support of the composition and/or providing a surface for bone and/or cartilage formation. The matrix may provide sequestering of the BMP protein or other cartilage/bone protein or other factors of the formulation and/or the appropriate environment for presentation of the formulation of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the cartilage and/or bone inductive proteins to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatability, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the formulations of the invention will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, poly(lactic acid), poly(glycolic acid) and polyanhydrides as well as coral. Other potential materials are biodegradable and biologically well defined, such as bone, tendon or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as poly(lactic acid) and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

The formulations of the invention may be employed in methods for treating a number of bone and/or cartilage defects, and periodontal disease. They may also be employed in methods for treating various types of wounds and in tissue repair. These methods, according to the invention, entail administering a composition of the invention to a patient needing such bone and/or cartilage formation, wound healing or tissue repair. The method therefore involves administration of a lysine analogue or other antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive protein in a pharmaceutically acceptable vehicle. These methods may further include the administration of the EACA and cartilage and/or bone inductive protein in conjunction with other growth factors including EGF, FGF, TGF-α, TGF-β, and PDGF.

A formulation of the present invention, which induces cartilage and/or bone formation in circumstances where bone and/or cartilage is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. A preparation employing lysine analogues such as EACA, serine protease inhibitors or other antifibrinolytic agents and a cartilage and/or bon inductiv protein may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. Formulations of the invention may be used in the treatment of periodontal disease, and in other tooth repair processes. Such formulations may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells.

The formulations of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. See, e.g. PCT Publication WO84/01106 for discussion of wound healing and related tissue repair.

The preparation of such physiologically acceptable formulations, having due regard to pH, isotonicity, stability and the like, is within the skill of the art. The therapeutic formulations are also presently valuable for veterinary applications due to the apparent lack of species specificity in cartilage and bone growth factor proteins. Domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the proteins of the present invention.

The therapeutic method includes administering the formulation topically, systemically, or locally as an implant or device. When administered, the therapeutic formulation for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the formulation may desirably be encapsulated or injected in a viscous form for delivery to the site of cartilage and/or bone or tissue damage. Topical administration may be suitable for wound healing and tissue repair.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the formulation of the invention. Factors which may modify the action of the formulation include the amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the type or types of bone and/or cartilage proteins present in the composition. The addition of other known growth factors, such as EGF, PDGF, TGF-α, TGF-β, and IGF-I and IGF-II to the final composition, may also effect the dosage.

The concentration of EACA utilized in the sheep experiments described below is 10^{-3}M. EACA is expected to have a wide margin of safety when used locally because up to 30 grams per 24 h urs can be

administered systemically without toxicity. [Hemostasis and Thrombosis Basic Principles and Clinical Practice pp. 380-384 (1988); Stefanini et al, J. of Urology 143: 559-561 (1990)].

Progress can be monitored by periodic assessment of cartilage and/or bone growth and/or repair. The progress can be monitored, for example, using x-rays, histomorphometric determinations and tetracycline labeling.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

EXAMPLE I

Rosen Modified Sampath-Reddi Assay

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A., 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of a formulation of the invention comprising EACA and cartilage and/or bone inductive proteins. This modified assay is herein called the Rosen Assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. EACA (Amicar) is dissolved in water and added to 20 mg rat matrix wetted with 0.1% TFA and bone inductive protein. The controls include samples containing the bone inductive protein without the EACA. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. Glycolmethacrylate sections (1µm) are stained with Von Kossa and acid fuschin or toluidine blue to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and newly formed bone and matrix. Two scoring methods are herein described. In the first scoring method a score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone. The second scoring method (which hereinafter may be referred to as the modified scoring method) is as follows: three non-adjacent sections are evaluated from each implant and averaged. "+/-" indicates tentative identification of cartilage or bone; "+1" indicates >10% of each section being new cartilage or bone; "+2", >25%; "+3", >50%; "+4", ~75%; "+5", >80%. The scores of the individual implants are tabulated to indicate assay variability.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the space occupied by the matrix. The implants containing rat matrix to which specific amounts of "BMP" protein or EACA and "BMP" protein have been added are removed from rats after approximately seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated and scored.

EXAMPLE II

Biological Activity of EACA and BMP-2 in the Rosen Assay

The efficacy of formulations of the invention comprising EACA and BMP-2 is tested in the Rosen Assay described above. The experiments include BMP-2, EACA and autologous blood, as well as BMP-2 and autologous blood without EACA. Negative controls without rat matrix include BMP-2 alone; BMP-2 and autologous blood and BMP-2 with autologous blood and EACA. EACA is dissolved in water and added to the rat matrix wetted within 0.1% TFA and BMP-2 [described in International Publication WO88/00205; PNAS, (USA) 87:2220-2224 (March 1990)]. Amounts of EACA ranging from 13 µg to 6.9 mg are added per 20 mg of rat demineralized, guanidinium chloride extracted bone or from 1 mM to 0.5 M based on a volume of 100 µg per implant. Samples are frozen and lyophilized and implanted in rats for seven days. The modified scoring method is utilized. In experiments containing autologous blood BMP-2 is mixed with the EACA and lyophilized in a siliconized glass tube. 100-200 ul of autologous blood is removed from the rat by orbital puncture and then added to the tube. The blood is allowed to clot for 1-2 hours; when firm, the clot is removed and implanted subcutaneously.

The amount of bone and/or cartilage formed increases with the use of EACA compared to samples lacking EACA when the same amount of cartilage/bone protein is utilized. The control samples do not result in any bone and/or cartilage formation. More specifically, in the presence of rat matrix, EACA increases the amount of bone formed as compared to BMP-2 without EACA at a moderate dose. EACA increases the amount of cartilage seen at a very low dose. Higher amounts of BMP-2 result in an increase of the amount of cartilage and bone (summed) formed and a more rapid appearance of bone.

Blood as a matrix dramatically increases the sensitivity of BMP-2 as compared to BMP-2 in the absence of blood. The recovery of any implant at all is quite small,

in the absence of blood. The omission of EACA in thes implants results in a lower recovery of activity and implants.

EXAMPLE III

Biological Activity of EACA and BMP-2 in a Sheen Model

An osteoperiosteal defect is created by excising a 2.5 cm midshaft segment from the right femur of skeletally mature sheep. Marrow contents and periosteum are removed from the exposed ends and the 2.5 cm gap stabilized with an anteriolateral metal fixation plate. Different materials are used to fill the gap in each of four groups of animals: (1) autologous bone graft from the cortical bone and iliac crest; (2) no implant ; (3) recombinant human BMP-2, described above, mixed with inactive bone matrix comprising ground sheep bone demineralized and extracted with guanidinium chloride and sterilized, plus EACA ; and (4) inactive bone matrix and EACA. The highly purified human BMP-2, expressed in mammalian cells, reconstituted with inactive bone matrix is frozen and lyophilized. Following lyophilization blood is added and EACA added to a final concentration of 1 mM.

Femoral radiographs are performed weekly. Animals are sacrificed at 12 weeks post-op, and biomechanical testing [four-point bending to failure] followed by histological analysis, is performed on the limbs.

The untreated defect [group (2)] and the defect treated with inactive matrix [group (4)] failed to show radiographic healing by week 12. All defects treated with BMP-2 [group (3)] showed radiographic evidence of new bone formation beginning at week 5 and progressing to union by week 12. Defects treated with autologous graft [group (1)] also show union by week 12. The radiographic findings were confirmed by gross analysis: specimens from groups (2) and (4) examined at 12 weeks showed gross motion at a fibrous tissue seam across the segmental defect site, while the autologous graft and the BMP-2 treated sites were rigid. Biomechanical testing supported these results. At week 12, the average bending strength [expressed as a percentage of the contralateral intact femur] was 111% for autologous graft, 16% for groups (2) & (4), and 91% for defects treated with BMP-2. Histologic analysis of a defect treated with BMP-2 showed evidence of new endochondral bone formation at two weeks post-op.

EXAMPLE IV

Rat Orthotopic Model

A 5 mm osteoperiosteal segmental defect (2x diaphyseal diameter) is created in the mid shaft of the femur of 325-350 gm Sprague-Dawley rats. Internal fixation is achieved with a four hole polyethylene plate fixed with 0.062 mm threaded Kirschner wires. Marrow is flushed from the intramedullary cavity at each side of the osteotomy. Two separate experiments using the rat orthotopic model are described below.

A. Three groups of fifteen rats are studied as follows:

Group I:	A rat matrix is implanted into the defect as a control.
Group II:	A rat matrix, EACA and 1 µg of BMP-2 are implanted as the low test dose.
Group III:	A rat matrix, EACA and 8 µg of BMP-2 are implanted as the high test dose.

EACA is added to 1 mM final concentration (assuming a volume of 50 ul) at the same time as the BMP-2 to the rat demineralized, guanidinium chloride-extracted bone. The sample is frozen and lyophilized. All rats are evaluated on day 7 for angiogenesis effect using dynamic quantitative bone scanning via intracardiac injection. The ratio of the total counts recorded over 60 seconds of the operated femur to the normal femur was then determined for each rat.

Bone formation in all rats is evaluated with serial radiographs taken at 1,2,3,4,5,6 and 9 weeks. The area of the defect occupied by bone is estimated by planimetry on lateral radiographs and recorded for each rat (as a percent of total defect area).

One rat is sacrificed each week for histologic analysis. Tissue from the grafted area and its surrounding bone is excised, decalcified, sectioned and stained with hematoxylin and eosin. Histological findings are recorded for sections taken parallel to the longitudinal axis of the bone extending over the entire length of the defect.

Those rats in which union occurrs across the defect are subjected to mechanical torsion testing to failure to determine the maximum torque, angular displacement, energy absorption and stiffness of the operated femur with the results compared to the contralateral normal femur.

B. Four groups of rats are studied as follows:

Group I:	BMP-2 and autologous blood are implanted into the defect.
Group II:	BMP-2, autologous blood and EACA are implanted into the defect.
Group III:	A PLGA matrix, BMP-2 and autologous blood are implanted in the defect.
Group IV:	A PLGA matrix, BMP-2, autologous blood and EACA are implanted in the defect.

The amount of BMP-2 is 15µg, 5µg and 1.5µg with 0.5M Arg in imidazole buffer pH 6.5. The amount of EACA per implant is 10mM. The BMP-2 and EACA are added to 56µl autologous blood. After mixing, the blood solution is added to the PLGA particles (80µl, ca.24mg.)

described below and thoroughly mixed. After 90-120 minutes the clot is implanted in the rat femur gap.

The porous PLGA particles [a 50:50 (molar) random copolymer of lactic acid and glycolic acid (PLGA) having an average molecular weight of 30-40K, a number average molecular weight of about 20K (by GPC relative to polystyrene standards), and an inherent viscosity of 0.35-0.45 dL/g] are prepared by a solvent evaporation technique. PLGA is dissolved in CH_2Cl_2 (15% w/v), and porosigen is suspended in this solution. The resulting solution is added to an excess poly(vinyl alcohol) aqueous solution (0.1% w/v). After a few hours of stirring, the particles are hardened in excess cold ethanol (95%). The resulting particles are washed with WFI and lyophilized to give a free-flowing product.

The rats are sacrificed after nine weeks. Ex-vivo analysis of the new bone is performed radiographically relative to the contralateral femur. Preliminary results taken after three weeks indicate that the presence of EACA at marginal (5µg) and low (1.5µg) doses of BMP-2 appears to increase the rate of unions observed as compared to the controls without EACA. Preliminary data indicates a lesser effect of the EACA at higher doses (15µg) of BMP-2 than with the lower doses. EACA particularly appears to increase the response in the BMP-2/autologous blood implants.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. A pharmaceutical formulation comprising an antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle.

2. The formulation of claim 1 wherein said antifibrinolytic agent is selected from the group consisting of lysine analogues and serine protease inhibitors.

3. A pharmaceutical formulation comprising epsilon amino caproic acid and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle.

4. The composition of claim 1 wherein said cartilage and/or bone inductive is selected from the group consisting of BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7.

5. The formulation of any one of claims 1 to 4 further comprising autologous blood.

6. The formulation of claim 1 further comprising a pharmaceutically acceptable matrix.

7. Th formulation f claim 1 further comprising a growth factor selected from the group consisting of IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α and TGF-β.

8. A pharmaceutical formulation comprising BMP-2, epsilon amino caproic acid, autologous blood and a PLGA matrix.

9. A method of formulating an osteoinductive preparation by combining epsilon amino caproic acid with at least one osteoinductive factor.

10. The method of claim 9 wherein said osteoinductive factor is selected from the group consisting of BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7.

11. Use of a lysine analogue and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle for the preparation of a pharmaceutical composition for the treatment of cartilage and/or bone defects.

12. The use of claim 11 wherein said lysine analogue is epsilon amino caproic acid.

13. The pharmaceutical formulation of claim 2 wherein said serine protease inhibitor is selected from the group consisting of aprotinin, α_2 antiplasmin and α_2 macroglobulin.

Claims for the following Contracting States : ES, GR

1. A method for the preparation of a pharmaceutical formulation comprising combining an antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive factor with a pharmaceutically acceptable vehicle.

2. The method of claim 1 wherein said antifibrinolytic agent is selected from the group consisting of lysine analogues and serine protease inhibitors.

3. A method for the preparation of a pharmaceutical formulation comprising combining epsilon amino caproic acid and a therapeutically effective amount of a cartilage and/or bone inductive factor with a pharmaceutically acceptable vehicle.

4. The method of claim 1 wherein said cartilage and/or bone inductive factor is selected from the group consisting of BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7.

5. The method of any one of claims 1 to 4 wherein the formulation further comprises autologous blood.

6. The method of claim 1, wherein the formulation further comprises a pharmaceutically acceptable matrix.

7. The method of claim 1, wherein the formulation further comprises a growth factor selected from the group consisting of IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α and TGF-β.

8. A method for the preparation of a pharmaceutical formulation comprising combining BMP-2, epsilon amino caproic acid, autologous blood and a PLGA matrix.

9. A method of formulating an osteoinductive preparation by combining epsilon amino caproic acid with at least one osteoinductive factor.

10. The method of claim 9 wherein said osteoinductive factor is selected from the group consisting of BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7.

11. Use of a lysine analogue and a therapeutically effective amount of a cartilage and/or hone inductive factor in a pharmaceutically acceptable vehicle for the preparation of a pharmaceutical composition for the treatment of cartilage and/or bone defects.

12. The use of claim 11 wherein said lysine analogue is epsilon amino caproic acid.

13. The method of claim 2, wherein said serine protease inhibitor is selected from the group consisting of aprotinin, α_2 antiplasmin and α_2 macroglobulin.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Arzneimittel, umfassend ein antifibrinolytisches Mittel und eine therapeutisch wirksame Menge eines eine Knorpel und/oder Knochen betreffende Reaktion hervorrufenden Faktors in einem pharmazeutisch verträglichen Träger.

2. Arzneimittel nach Anspruch 1, wobei das antifibrinolytische Mittel ausgewählt ist aus Lysinanalogen oder Serinproteaseinhibitoren.

3. Arzneimittel, umfassend ε-Aminocapronsäure und eine therapeutisch wirksame Menge eines eine Knorpel und/oder Knochen betreffende Reaktion hervorrufenden Faktors in einem pharmazeutisch verträglichen Träger.

4. Arzneimittel nach Anspruch 1, wobei der eine Knorpel und/oder Knochen betreffende Reaktion hervorrufende Faktor ausgewählt ist aus BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 oder BMP-7.

5. Arzneimittel nach inem der Ansprüche 1 bis 4, außerdem autologes Blut umfassend.

6. Arzneimittel nach Anspruch 1, außerdem eine pharmazeutisch verträgliche Matrix umfassend.

7. Arzneimittel nach Anspruch 1, außerdem einen Wachstumsfaktor, ausgewählt aus IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α oder TGF-β, umfassend.

8. Arzneimittel, umfassend BMP-2, ε-Aminocapronsäure, autologes Blut und eine PLGA-Matrix.

9. Verfahren zur Formulierung eines eine Knochen betreffende Reaktion hervorrufenden Arzneimittels durch Kombinieren von ε-Aminocapronsäure mit mindestens einem eine Knochen betreffende Reaktion hervorrufenden Faktor.

10. Verfahren nach Anspruch 9, wobei der eine Knochen betreffende Reaktion hervorrufende Faktor ausgewählt ist aus BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 oder BMP-7.

11. Verwendung eines Lysinanalogen und einer therapeutisch wirksamen Menge eines eine Knorpel und/oder Knochen betreffende Reaktion hervorrufenden Faktors in einem pharmazeutisch verträglichen Träger zur Herstellung eines Arzneimittels zur Behandlung von Knorpel- und/oder Knochendefekten.

12. Verwendung nach Anspruch 11, wobei das Lysinanaloge ε-Aminocapronsäure ist.

13. Arzneimittel nach Anspruch 2, wobei der Serinproteaseinhibitor ausgewählt ist aus Aprotinin, α_2-Antiplasmin oder α_2-Makroglobulin.

Patentansprüche für folgende Vetragsstaaten : ES, GR

1. Verfahren zur Herstellung eines Arzneimittels, umfassend das Kombinieren eines antifibrinolytischen Mittels und einer therapeutisch wirksamen Menge eines eine Knorpel und/oder Knochen betreffende Reaktion hervorrufenden Faktors mit einem pharmazeutisch verträglichen Träger.

2. Verfahren nach Anspruch 1, wobei das antifibrinolytische Mittel ausgewählt ist aus Lysinanalogen oder Serinproteaseinhibitoren.

3. Verfahren zur Herstellung eines Arzneimittels, umfassend das Kombinieren von ε-Aminocapronsäure und einer therapeutisch wirksamen Menge eines eine Knorpel und/oder Knochen betreffende Reaktion hervorrufenden Faktors mit einem pharmazeutisch verträglichen Träger.

4. Verfahren nach Anspruch 1, wobei der eine Knorpel und/oder Knochen betreffende Reaktion hervorru-

fende Faktor ausgewählt ist aus BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 oder BMP-7.

5. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Arzneimittel außerdem autologes Blut umfaßt.

6. Verfahren nach Anspruch 1, wobei das Arzneimittel außerdem eine pharmazeutisch verträgliche Matrix umfaßt.

7. Verfahren nach Anspruch 1, wobei das Arzneimittel außerdem einen Wachstumsfaktor, ausgewählt aus IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α oder TGF-β, umfaßt.

8. Verfahren zur Herstellung eines Arzneimittels, umfassend das Kombinieren von BMP-2, ε-Aminocapronsäure, autologem Blut und einer PLGA-Matrix.

9. Verfahren zur Formulierung eines eine Knochen betreffende Reaktion hervorrufenden Arzneimittels durch Kombinieren von ε-Aminocapronsäure mit mindestens einem eine Knochen betreffende Reaktion hervorrufenden Faktor.

10. Verfahren nach Anspruch 9, wobei der eine Knochen betreffende Reaktion hervorrufende Faktor ausgewählt ist aus BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 oder BMP-7.

11. Verwendung eines Lysinanalogen und einer therapeutisch wirksamen Menge eines eine Knorpel und/oder Knochen betreffende Reaktion hervorrufenden Faktors in einem pharmazeutisch verträglichen Träger zur Herstellung eines Arzneimittels zur Behandlung von Knorpel- und/oder Knochendefekten.

12. Verwendung nach Anspruch 11, wobei das Lysinanaloge ε-Aminocapronsäure ist.

13. Verfahren nach Anspruch 2, wobei der Serinproteaseinhibitor ausgewählt ist aus Aprotinin, α_2-Antiplasmin oder α_2-Makroglobulin.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Formulation pharmaceutique comprenant un agent antifibrinolytique et une quantité thérapeutiquement efficace d'un facteur cartilagino- et/ou ostéoinductif dans un véhicule pharmaceutiquement acceptable.

2. Formulation suivant la revendication 1, dans laquelle l'agent antifibrinolytique est choisi dans le groupe comprenant les analogues de lysine et les inhibiteurs de sérine protéase.

3. Formulation pharmaceutique comprenant de l'acide epsilon amino caproïque et une quantité thérapeutiquement efficace d'un facteur cartilagino- et/ou ostéoinductif dans un véhicule pharmaceutiquement acceptable.

4. Composition suivant la revendication 1, dans laquelle le facteur cartilagino- et/ou ostéoinductif est choisi dans le groupe comprenant les BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 et BMP-7.

5. Formulation suivant l'une quelconque des revendications 1 à 4, comprenant de plus du sang autologue.

6. Formulation suivant la revendication 1, comprenant de plus une trame pharmaceutiquement acceptable.

7. Formulation suivant la revendication 1, comprenant de plus un facteur de croissance choisi dans le groupe comprenant les IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α et TGF-β.

8. Formulation pharmaceutique comprenant de la BMP-2, de l'acide epsilon amino caproïque, du sang autologue et une trame de PGLA.

9. Procédé de formulation d'une préparation ostéoinductive par la combinaison d'acide epsilon amino caproïque avec au moins un facteur ostéoinductif.

10. Procédé suivant la revendication 9, dans lequel le facteur ostéoinductif est choisi dans le groupe comprenant les BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 et BMP-7.

11. Utilisation d'un analogue de lysine et d'une quantité thérapeutiquement efficace d'un facteur cartilagino- et/ou ostéoinductif dans un véhicule pharmaceutiquement acceptable pour la préparation d'une composition pharmaceutique pour le traitement de défauts cartilagineux et/ou osseux.

12. Utilisation suivant la revendication 11, dans laquelle l'analogue de lysine est de l'acide epsilon amino caproïque.

13. Formulation pharmaceutique suivant la revendication 12, dans laquelle l'inhibiteur de sérine protéase est choisi dans le groupe comprenant l'aprotinine, l'α_2 antiplasmine et l'α_2 macroglobuline.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé pour la préparation d'un formulation pharmaceutique comprenant la combinaison d'un agent antifibrinolytique et d'une quantité thérapeutiquement efficace d'un facteur cartilagino- et/ou ostéoinductif avec un véhicule pharmaceutiquement acceptable.

2. Procédé suivant la revendication 1, dans lequel l'agent antifibrinolytique est choisi dans le groupe comprenant les analogues de lysine et les inhibiteurs de sérine protéase.

3. Procédé pour la préparation d'une formulation pharmaceutique comprenant la combinaison d'acide epsilon amino caproïque et d'une quantité thérapeutiquement efficace d'un facteur cartilagino- et/ou ostéoinductif avec un véhicule pharmaceutiquement acceptable.

4. Procédé suivant la revendication 1, dans lequel le facteur cartilagino- et/ou ostéoinductif est choisi dans le groupe comprenant les BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 et BMP-7.

5. Procédé suivant l'une quelconque des revendications 1 à 4, dans lequel la formulation comprend de plus du sang autologue.

6. Procédé suivant la revendication 1, dans lequel la formulation comprenant de plus une trame pharmaceutiquement acceptable.

7. Procédé suivant la revendication 1, dans lequel la formulation comprenant de plus un facteur de croissance choisi dans le groupe comprenant les IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α et TGF-β.

8. Procédé pour la préparation d'une formulation pharmaceutique comprenant la combinaison de BMP-2, d'acide epsilon amino caproïque, de sang autologue et d'une trame de PGLA.

9. Procédé de formulation d'une préparation ostéoinductive par la combinaison d'acide epsilon amino caproïque avec au moins un facteur ostéoinductif.

10. Procédé suivant la revendication 9, dans lequel le facteur ostéoinductif est choisi dans le groupe comprenant les BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 et BMP-7.

11. Utilisation d'un analogue de lysine et d'une quantité thérapeutiquement efficace d'un facteur cartilagino- et/ou ostéoinductif dans un véhicule pharmaceutiquement acceptable pour la préparation d'une composition pharmaceutique pour le traitement de défauts cartilagineux et/ou osseux.

12. Utilisation suivant la revendication 11, dans laquelle l'analogue de lysine est de l'acide epsilon amino caproïque.

13. Procédé suivant la revendication 2, dans lequel l'inhibiteur de sérine protéase est choisi dans le groupe comprenant l'aprotinine, l'α_2 antiplasmine et l'α_2 macroglobuline.